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A comparative study of solvent and supercritical CO_2 extraction of *Simarouba gluaca* seed oil

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SUMMARY: In the present study, the supercritical carbon dioxide (CO_2) extraction of oil from *Simarouba gluaca* seeds was carried out at varying conditions of pressure (300–500 bar), temperature (50–70 °C) and CO_2 flow rate (10–30 g·min^{-1}). The extraction condition for maximum oil yield was obtained at 500 bar pressure, 70 °C and at 30 g·min^{-1} flow rate of CO_2. The extracted oil was analyzed thoroughly for physico-chemical properties and compared with those of conventional solvent extracted oil. An interesting observation is a significant reduction in the phosphorus content of the oil (8.4 mg·kg^{-1}) extracted using supercritical CO_2 compared to the phosphorous content of the solvent extracted oil (97 mg·kg^{-1}). Moreover, the content of total tocopherols in supercritically extracted oil (135.6 mg·kg^{-1}) was found to be higher than the solvent extracted oil (111 mg·kg^{-1}). The rest of the physico-chemical properties of the two differently extracted oils matched well with each other. The results indicated the possible benefits of supercritical CO_2 extraction over solvent extraction of *Simarouba gluaca* seed oil.

KEYWORDS: *Extraction; Physico-chemical Characterizations;* Simarouba gluaca *seeds; Supercritical CO_2*

RESUMEN: ***Estudio comparativo de la extracción con solvente y CO_2 supercrítico de aceites de semillas de* Simarouba glauca**. En el presente estudio se llevó a cabo la extracción con dióxido de carbono supercrítico (CO_2) de aceites de semillas de *Simarouba gluaca* en diversas condiciones de presión (300–500 bar), temperatura (50–70 °C) y el caudal de CO_2 (10–30 g·min^{-1}). La condición de extracción para obtener el máximo rendimiento de aceite se obtuvo a una presión de 500 bares, una temperatura de 70 °C y un caudal de CO_2 de 30 g·min^{-1}. Al aceite extraido se determinó sus propiedades fisicoquímicas y se comparó con las del aceite extraído con disolvente convencional. Una observación interesante es la reducción significativa del contenido de fósforo (8,4 mg·kg^{-1}) del aceite extraído utilizando CO_2 supercrítico en comparación con el del aceite extraído con disolvente (97 mg·kg^{-1}). Además, el contenido de tocoferol total en el aceite extraído supercríticamente (135,6 mg·kg^{-1}) fué más elevado que el extracto con disolvente (111 mg·kg^{-1}). El resto de las propiedades físico-químicas de dos aceites extraídos de forma diferente se equipararon entre sí. Los resultados indicaron los posibles beneficios de la extracción con CO_2 supercrítico sobre la extracción con disolvente del aceite de semilla *Simarouba gluaca*.

PALABRAS CLAVE: *Caracterización fisicoquímica; CO_2 supercrítico; Extracción; Semillas de* Simarouba gluaca

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1. INTRODUCTION

Simarouba gluaca, generally known as Paradise tree, Simba, Maruba, Aceituno, Pitomba and Laxmitharu in different parts of India, is mainly available in Cuba, Mexico, Haiti, Jamaica, Central America and the southern part of India. *Simarouba gluaca* belongs to the family of *Simaroubaceae* and it can easily grow under hostile ecological conditions. This evergreen tree grows up to 7–15 meters high with a trunk diameter of approximately 0.508 meters. The tree is economically attractive to industry as each part of it can be used for profitable purposes (Patil Manasi *et al.,* 2011). The seed kernel contains 55 to 65% crude oil and the purified oil is mostly used for cooking purposes in tropical countries due to the presence of rich amounts of vitamin A and vitamin E (Severen, 1953, Panhwar, 2007). The oil is composed of fatty acids like palmitic (11–12%), stearic (27–33%) and oleic (52–54%) as major fatty acids (Kureel *et al.,* 2009). The oil is suitable for the manufacture of soaps, detergents, paint, pharmaceuticals etc. Some authors have reported the preparation of biodiesel (Vijay kumar *et al.,* 2013; Mishra *et al.,* 2012; Hifjur *et al.,* 2013) and lubricants **(**Anjana *et al.,* 2013**)** with this oil. The oil contains nearly 30% monounsaturated symmetrical triglyceride with a high melting point ≈ 29 °C and hence can be used as a cocoa butter equivalent (Jeyarani *et al*., 2001). Commercially *Simarouba gluaca* oil is extracted using mechanical expellers or with a suitable solvent. The oil is refined in a similar fashion as other vegetable oils (Anil *et al.,* 2011).

An increase in consumer demand for natural products and strict government regulations on the use of organic solvents led to the adaptation of alternate processing technologies such as supercritical CO_2 extraction (Luthria, 2004; Passos *et al*., 2010). Supercritical CO_2 extraction is a growing separation technology in industrial operations to extract the desired components from its source. It is non toxic and eco-friendly process for extracting oil from its sources especially if the oil is rich in nutrients and has commercial importance for use in different food applications.

Simarouba gluaca oil, which has a high potential to be used as a cocoa butter equivalent has not been explored to be extracted using supercritical CO_2. In the present study, an attempt has been made to study the effect of various processing conditions for the supercritical CO_2 extraction of oil from *Simarouba gluaca* seeds. Extraction studies were carried out by varying extraction pressures, temperatures and CO_2 flow rates. In an effort to understand the efficiency of supercritical extraction and also for a comparative evaluation of the physicochemical properties of the extracted oil, traditional solvent extraction of the oil was also performed.

2. MATERIALS AND METHODS

2.1. Materials

Simarouba gluaca seeds were supplied by the AP Forest Department, Hyderabad, India. All the chemicals and solvents used in this study were purchased from M/s. Sd fine Chem. (Mumbai, India) and were of high purity.

2.2. Methods

2.2.1. Extraction of oil

The dried *Simarouba gluaca* seeds were ground to powder and the oil was extracted in a Soxhlet apparatus using hexane. The oil content was determined as a percentage of the extracted oil from the sample weight (w/w). The extracted oil was stored at 4 °C in a glass bottle under a nitrogen blanket for further analysis.

2.2.2. Supercritical CO_2 extraction

The oil from the powdered *Simarouba gluaca* seeds was extracted using a supercritical CO_2 extractor (SFE 500 mL) supplied by Waters Corporation, Milford, USA. The unit is comprised of a chiller, CO_2 pump, co-solvent pump, heat exchanger, extraction vessel, ABPR (automatic back pressure regulator), fraction collector, etc. The system is designed to withstand pressures of up to 600 bar and temperatures up to 80 °C. The volume of the extraction vessel is 500 mL and the system is controlled by PLC. Initially, 150 g of seed sample were taken in a 0.45 µm cotton filter bag and inserted into the extraction vessel and then the chiller temperature was brought down to 5 °C. The CO_2 filled cylinder was connected to the unit. The CO_2 gas was pre-liquefied by passing through the shell-side heat exchanger and then pumped into extraction vessel where the temperature and pressure were maintained at above critical conditions. The supercritical state of CO_2 was achieved at this stage. The oil extraction was carried out at different operating conditions of pressure and temperature. Each experiment was run for obtaining the maximum possible oil. The extraction time was set at 8 hours. Oil was then collected in a sample bottle from the collection vessel after each run and weighed to get the oil yield. The sample bottle was tightly sealed and kept in a refrigerator for further analysis. Three different oil samples were obtained for each extraction condition and the parameters were measured for each one.

2.2.3. Physico-chemical characteristics

The physico-chemical characteristics of the extracted oils were measured and analyzed according to the well-established standard methods.

The parameters such as Acid value (AOCS 1994 Ca 5a-40), Iodine value (AOCS 1994 Cd 1-25), Refractive Index (AOCS 1994 Cc 7-25), Saponification value (AOCS 1994 Cd 3-25), Moisture and volatiles (AOCS 1994 Ca 2c-25), Unsaponifiable matter (AOCS 1994 Ca 6a-40), Phosphorous (Pacquot et al. 1987) , Peroxide value (AOCS 1994 Cd 8-53), were analyzed. Density was measured according to the ASTM D 4052 method (ASTM 1984). Color was measured as per the AOCS method using the Lovibond Tintometer (Lovibond model PFX 995) (AOCS 1994 Cc 13e-92). Kinematic viscosity (cSt) was measured as per the ASTM standard method D-445 (cannon instrument Co., state college, PA) (ASTM 1970).

2.2.4. High performance liquid chromatography

High performance liquid chromatography (HPLC) was used for analyzing the tocopherol and tocotrienol contents of the oil samples according to the AOCS procedure (AOCS 1994 Ce 8-89). The analysis was performed on an Agilent 1100 series HPLC unit equipped with a fluorescence detector. The separation of tocopherols and tocotrienols was performed by using a normal phase silica column (LiChroshper Si-60; 250 mm × 4.0 mm × 5 µm) (Merck Millipore, UK). The excitation and emission wave length of the detector were set at 292 and 330 nm, respectively. The isocratic mobile phase consisting of hexane and isopropyl alcohol (99.5:0.5, v/v) was used at a flow rate of 1.0 ml/min. The total tocopherol and tocotrienol contents were expressed as $mg \cdot kg^{-1}$.

2.2.5. Gas chromatography

The fatty acid composition of the extracted oil was analyzed by gas chromatography (GC) after derivatization of the oil to its methyl ester. Fatty acid methyl esters (FAME) were prepared from the oil samples using the solution of methanolic sulphuric acid (2% v/v) (Christie 1982). The analysis was performed on an Agilent 6890 gas chromatograph which was assembled with a flame ionization detector (FID) and a capillary column (DB-225; 30 m × 0.25 mm × 0.25 µm). The oven temperatures were programmed for 2 min at 160 °C, raised to 230 °C at 5°C/min and finally held at 230 °C for 15 min. The injector and detector temperatures were set as 230 and 270 °C, respectively, with a split ratio of 10:1. The flow rate of the carrier gas, N_2 was maintained at 1 mL/min. The flow rates of air and hydrogen were 300 mL min^{-1} and 30 mL min^{-1}, respectively. The identification of fatty acid composition was recorded by comparing the retention times of standard FAMEs, C_4-C_{24} (Supelco, USA). Injection was performed in triplicate for each sample and average values are reported.

2.2.6. Determination of solid fat content by pulsed-NMR

The solid fat content was analyzed by following the AOCS official method Cd 16b-93 (AOCS 2004b). A minispec mq20 p-NMR analyzer (Bruker Corporation) was used to determine the SFC profiles of the oil samples.

2.3. Statistical analysis

The reported values of the physico-chemical characteristics are the means of the three replicates, presented as means ± standard deviations (SD) and they were also analyzed by a paired Student's t-test to evaluate the statistical significance.

3. RESULTS AND DISCUSSION

In the current investigation, soxhlet extraction of *Simarouba gluaca* seeds was carried out by employing hexane and the oil content in the seeds was found to be 57.2%. The physicochemical properties of the extracted oil were thoroughly analyzed and are given in Table 1. The composition of different fatty acids in the extracted oil was also analyzed by GC after derivatization and is shown in Table 2.

The extraction conditions for the supercritical CO_2 extraction of oil from *Simarouba gluaca* seeds were applied in the present study for the maximum possible extraction of oil. Different conditions such as pressure (300 to 500 bar), temperature (50 to 70 °C) and CO_2 flow rate (10 to 30 $g \cdot min^{-1}$) were varied. The run time of extraction was fixed at 8 hours, matching the run time of conventional solvent extraction. Initially, the supercritical CO_2 extraction was carried out at three different temperatures (50, 60 and 70 °C) and three different pressures (300, 400 and 500 bar) with a constant CO_2 flow rate of 10 $g \cdot min^{-1}$. The oil yields were found to be 4.2, 11.6 and 18.8% at 300, 400 and 500 bar, respectively at 50 °C. On increasing the temperature to 60 °C, there was a marginal increase in the extracted oil yields to 5.73, 14.0 and 22.6%, respectively at 300, 400 and 500 bar. With a further increase in extraction temperature to 70 °C, the yields increased to 6.3, 15.8 and 27%, respectively, at 300, 400 and 500 bar. Thus there exists an increasing trend in extracted oil yield with an increase in extraction temperature and pressure.

In an effort to study the effect of the flow rate of CO_2, the extraction of oil from *Simarouba gluaca* seeds was also carried out at increased flow rates of 20 and 30 $g \cdot min^{-1}$. At 50 °C and at a flow rate of 20 $g \cdot min^{-1}$, the oil yields recorded were 10.9, 22.2 and 29.1% at 300, 400 and 500 bar, respectively. On increasing the temperature to 60 °C and maintaining the same flow rate of CO_2, the extracted oil yields increased to 13.5, 26.2 and 35.8% at 300, 400 and 500 bar, respectively. However, a considerable

TABLE 1. Physico-chemical characteristics of Solvent and Supercritical Extracted *Simarouba glauca* oil

Characteristics	Solvent Extracted Oil	Supercritical CO_2 Extracted Oil*
Oil content (%)	57.2±0.26	57.0±0.9[a]
Moisture content (%)	0.18±0.01	0.16±0.01
FFA (%)	1.8±0.17	1.5±0.13[b]
Iodine value (g I_2 $g^{-1}\cdot10^{-2}$)	58.5±0.21	58.3±0.26[a]
Saponification value (mg KOH·g^{-1})	190.6±0.65	190.4±0.49[a]
Peroxide value (mEq $O_2\cdot kg^{-1}$)	1.8±0.36	1.0±0.08[a]
Unsaponifiable matter (%)	0.7±0.01	0.63±0.02[a]
Phosphorous content (mg·kg^{-1})	97.0±0.1	8.4 (±) 0.21[c]
Color in 1" cell (Y+5R)	36.6 (2.3R, 25.2Y)±0.05	33.0 (1.2R, 27.0Y)±0.06[d]
Density at 40 °C (g·cm^{-3})	0.897±0	0.895±0
Specific gravity at 40 °C	0.904±0	0.902±0
RI at 25 °C	1.455±0	1.455±0
Viscosity at 40 °C (cSt)	48.57±0.1	48.52±0.3[c]
Tocols (mg·kg^{-1})	111±0	135.6±0

** at 500 bar and 70 °C. Values are means ± SD for three samples. [a]Significantly different from solvent extracted Simarouba glauca seed oil: P < 0.05. [b]Significantly different from solvent extracted Simarouba glauca seed oil: P < 0.05. [c]Significantly different from solvent extracted Simarouba glauca seed oil: P > 0.001. [d]Significantly different from solvent extracted Simarouba glauca seed oil: P < 0.001.*

TABLE 2. Fatty Acid Composition of Solvent Extracted and Supercritical CO_2 Extracted Oil

Fatty Acid	Solvent Extracted Oil (%)	Supercritical CO_2 Extracted Oil* (%)
C16:0	11.8	10.8
C18:0	26.6	27.6
C18:1	56.6	57.4
C18:2	3.2	2.3
C18:3	0.2	0.3
C20:0	1.5	1.5
C20:1	0.1	0.1

**at 500 bar and 70 °C*

increase in extracted oil yield (16, 29.3 and 42.6% at 300, 400 and 500 bar, respectively) was observed upon further increase in extraction temperature to 70 °C at the same flow rate of g·min^{-1}. These results encouraged us to further increase the flow rate of CO_2 to 30 g·min^{-1}. At 50 °C and at a flow rate of 30 g·min^{-1}, the oil yields recorded were 25.9, 34.8 and 40.0% at 300, 400 and 500 bar pressures, respectively. At 60 °C, the yields of the extracted oil increased to 29.0, 42.6 and 55.0% at 300, 400 and 500 bar, respectively. Extraction temperature was raised further to the final optimum temperature of 70 °C while maintaining the flow rate of 30 g·min^{-1}. The percentages of extracted oil were found to be 30.0, 45.5 and 57.0% at 300, 400 and 500 bar respectively. The effect of temperature and pressure on oil yield at a constant CO_2 flow rate of 10, 20 and 30 g·min^{-1} are shown in figures 1, 2 and 3. Thus the maximum possible extraction of oil (57.0%) from *Simarouba gluaca* seeds was achieved at the extraction temperature of 70 °C, pressure 500 bar and flow rate of 30 g·min^{-1}. At the above set conditions of temperature, pressure and flow rate, another set of extraction was conducted in order to optimize the extraction time (2, 4, 6 and 8 hours). The results obtained indicate that the maximum oil yields of 57.0% could be achieved within 6 hours, beyond which no appreciable increase was observed. However, this study could not be extended beyond the studied temperature, pressure and flow rate because of the limitations of the system.

Table 1 depicts the physico-chemical properties of *Simarouba gluaca* oil extracted with supercritical CO_2 as well as using hexane. From this data, it is observed that the moisture content, % free fatty acids (FFA), iodine value, saponification value, unsaponifiable matter, color (in 1″ cell), density, refractive index and viscosity remained more or less constant for both solvent and supercritical CO_2 extracted oils. However, peroxide values showed a slight difference of 0.8 between supercritical CO_2 extracted and solvent extracted oil. This indicates that the quality of supercritical CO_2 extracted oil was slightly better when compared to solvent extracted oil. The content of tocols of the supercritical CO_2 extracted oil was more when compared to the solvent extracted oil. The values were 111 mg·$kg^{-1}$for solvent extracted oil and 135.6 mg·kg^{-1} for supercritical extracted oil. Earlier reports have shown very few physical properties of the oil (Anil *et al.,* 2011).

A major and important observation was the non-extractability of phospholipids in the oil extracted by supercritical CO_2 when compared

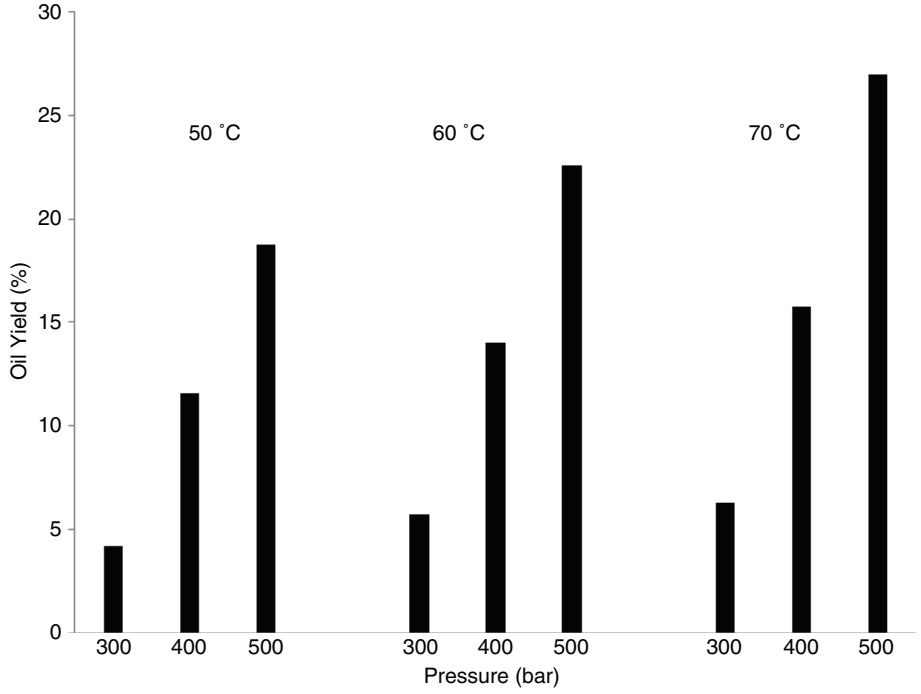

FIGURE 1. The effect of temperature and pressure on oil yield at CO_2 flow rate of 10 g·min^{-1}.

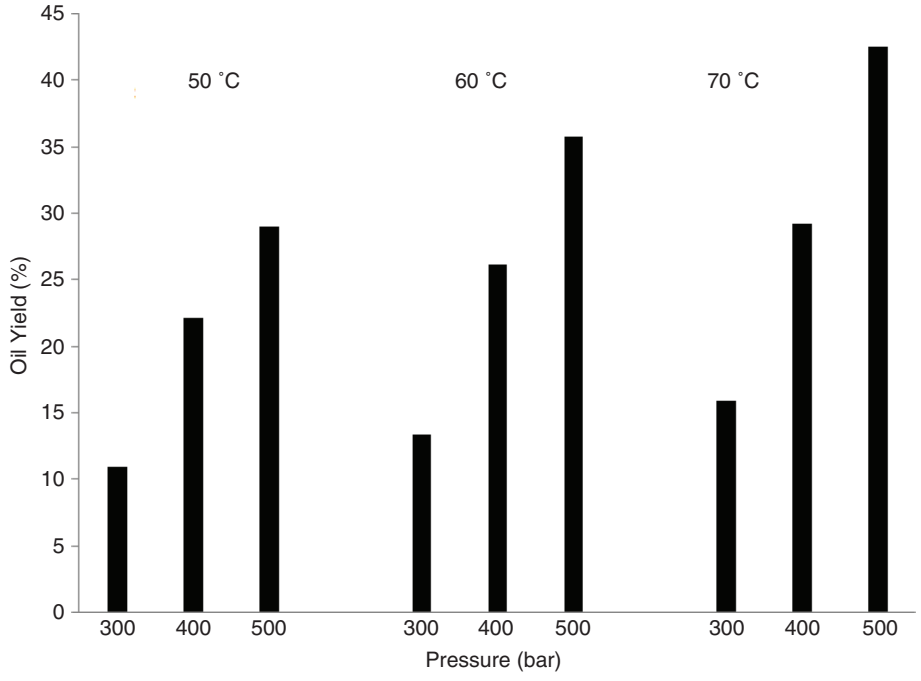

FIGURE 2. The effect of temperature and pressure on oil yield at CO_2 flow rate of 20 g·min^{-1}.

to the solvent extracted oil. During supercritical CO_2 extraction, the phospholipids do not get extracted into the oil. This is because of the non polar nature of the CO_2 which does not dissolve the phospholipids contained in the seeds which are otherwise polar in nature. The values were 97 and 8.4 mg·kg^{-1} for solvent and supercritical CO_2 extracted oils, respectively. Due to this reason, the

color of supercritical CO_2 extracted oil was light yellow and was better in appearance when compared to the solvent extracted oil. Lesser amount of phosphorous indicates that de-gumming, which is an important processing operation in vegetable oil processing, can be avoided when the oil is extracted using supercritical CO_2.

The fatty acid composition of both the extracted oils is almost similar and is shown in Table 2 and the respective chromatograms are shown in figures 4 and 5. The oil contained 39.9% saturated fatty acids and 60.1% unsaturated fatty acids. Table 3 gives the solid fat content of solvent and supercritical CO_2 extracted oils. The melting profiles indicate that both the oils have sharp melting points at 35 °C.

4. CONCLUSION

In this study, the supercritical CO_2 extraction of *Simarouba gluaca* seed oil at varying conditions of pressure (300–500 bar), temperature (50–70 °C) and CO_2 flow rate (10 to 30 g·min^{-1}) was carried out for optimal yield. The maximum yield, 57% was obtained at 500 bar, 70 °C and a CO_2 flow rate of 30 g·min^{-1}. Solvent extracted and supercritical CO_2 extracted oils were thoroughly characterized for physico-chemical properties. It was found that the phosphorous content of the supercritical CO_2 extracted oil had a much smaller amount of phosphorous compared to the solvent extracted oil. Total tocol contents were also higher in supercritical CO_2 extracted oil. The other parameters almost

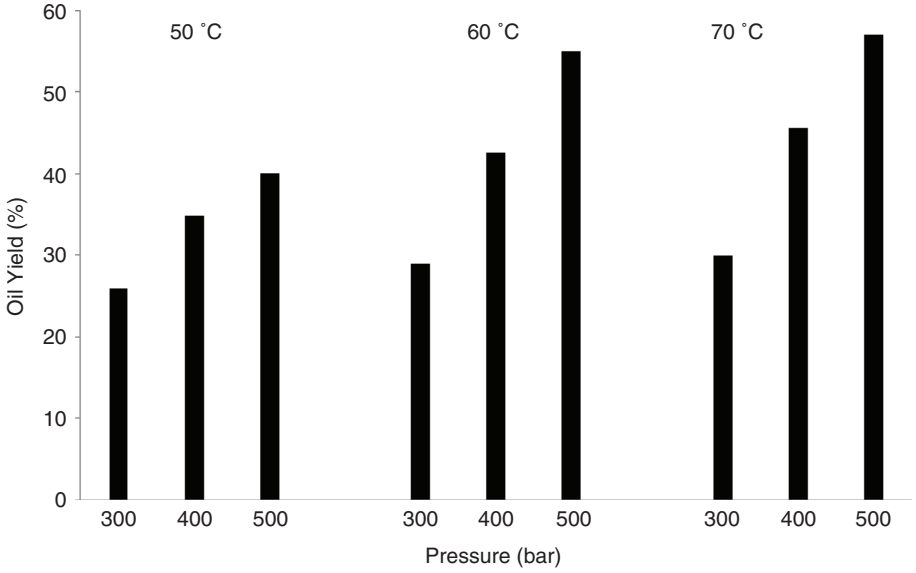

FIGURE 3. The effect of temperature and pressure on oil yield at CO_2 flow rate of 30 g·min^{-1}.

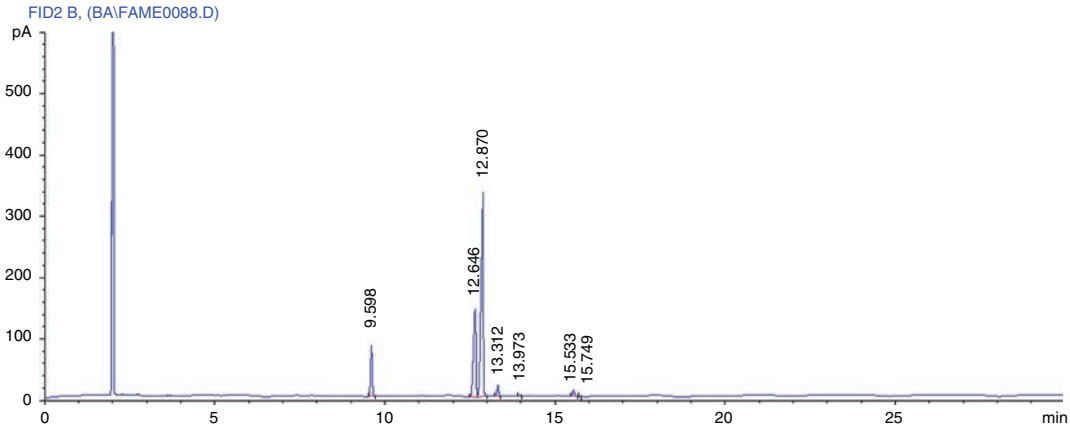

FIGURE 4. GC chromatogram of fatty acid methyl ester of supercritical fluid extracted *Simarouba gluaca* seed oil.

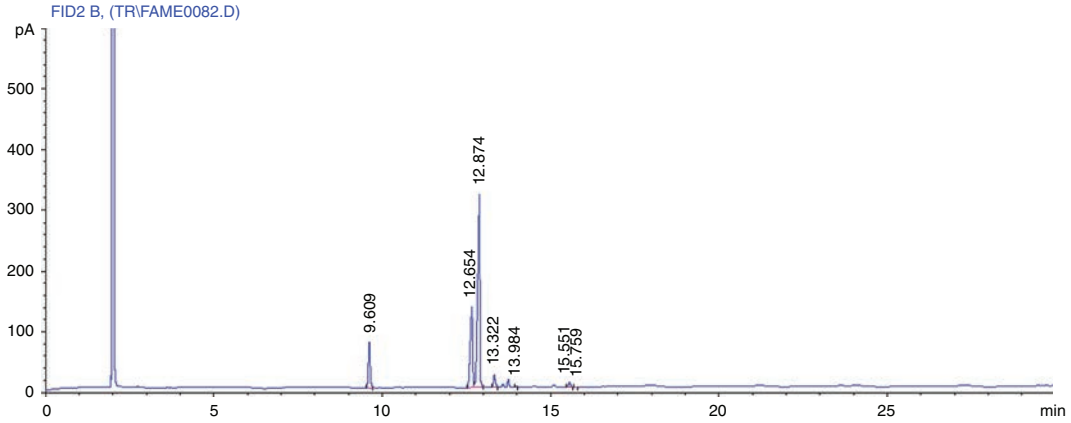

FIGURE 5. GC chromatogram of fatty acid methyl ester of solvent extracted *Simarouba gluaca* seed oil.

TABLE 3. Solid Fat Content (in %) of Solvent and Supercritical CO_2 Extracted *Simarouba gluaca* oils

	Solid Fat Content (%)	
Temp (°C)	Solvent Extracted Oil	Supercritical CO_2 Extracted Oil*
0	68.60	67.90
5	62.01	62.80
10	58.95	57.83
15	46.60	47.28
20	25.91	25.49
25	16.50	15.86
30	5.46	5.21
35	0	0

**at 500 bar and 70 °C*

remained the same for both supercritical CO_2 and solvent extracted oil.

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